WHAT IS CLAIMED IS:

1. An array reader (110) suitable for clinical purposes for reading a two-dimensional array (103) of features on a planar substrate (302), in which the features carry photo-responsive markers, the markers capable of emitting light upon excitation, the array reader comprising:

an illumination system (120) for simultaneously exciting multiple photo-responsive markers distributed in a two-dimensional array over the substrate,

and an image collection and recording system (140) having a field of view for emissions from the features on the substrate,

wherein the illumination system (120) comprises a light source arranged to flood the two-dimensional array (103) with light at an excitation wavelength, along an illumination path (P) disposed at an angle (θ) between about 20 and 50° to the plane of the substrate,

the image collection and recording system (140) having an image-acquiring axis (141) substantially normal to the plane of the substrate (102) carrying the array, employing a two-dimensional sensor (146) comprising a solid-state array (203, Fig. 4) of photosensitive elements , e.g. a charge-coupled device (CCD) or a CMOS array, and the image collection and recording system constructed and arranged to apply an image of the array of features upon the solid-state array of size (Dc) of the same order of magnitude as the size D_o of the array, e.g. within a range of magnification of up to about 25% or reduction down to 75%, the image collection and recording system (140) having an intermediate numerical aperture NA to enable recording the image of fluorescence from the excited two-dimensional array with clinical accuracy and without translation of the array.

2. The array reader of claim 1 in which the image collection and recording system (140) has its nearest component spaced (dimension h) at least 5 mm, preferably at least 10 mm, from the substrate (102) or its support, the component constructed and arranged to provide space below said component for said illumination path (P) to the two-dimensional array on said substrate.

3. The array reader of claim 1 or 2 in which the image collection and recording system has an effective aperture between NA=0.3 and NA=0.60, preferably the value of NA being between about 4.0 and 5.5.

4. The array reader of claim 1, 2 or 3 in which the image collection and recording system (140) has a field of view (V Fig. 1, Fig. 7B) on the substrate of area between about 50 mm^2 and 300 mm^2.

5. The array reader of any of the foregoing claims in which said illumination system comprises one or more light-emitting diodes (122).

6. The illumination system of claim 5 constructed and arranged to provide excitation illumination over the two-dimensional array (103) on the substrate (102) of a power density greater than 30 mW/cm^2.

7. The array reader of claim 6 or 7 in which said image collection and recording system (140) includes a timer that is cooperatively related to the illumination system to provide exposure sufficient to produce a fluence of excitation radiation at said substrate greater than about 15 mJ/cm^2 across said two-dimensional array.

8. The array reader of any of the foregoing claims in which the field of view (V) of the array reader has a diameter (D_o) of the order of 10 mm or more.

9. The array reader of any of the foregoing claims in which a spot (166, 202) of the array of features is imaged onto at least of 50 pixel elements of the solid state array (203), for example upon CCD or CMOS elements.

10. The array reader of any of the foregoing claims constructed and arranged to deliver to said solid state sensor array (203) an image of the field of view that is not magnified.

11. The array reader of any of the foregoing claims constructed and arranged to deliver to said solid state sensor array an image of the field of view reduced between about 30% and 50%.

12. The array reader of any of the foregoing claims constructed and arranged to image a two-dimensional array (103) of at least 100 spots (202) each of diameter at least about 80 micron, preferably at least about 100 micron diameter.

13. The array reader of any of the foregoing claims constructed and arranged to produce during a single imaging interval an image of an array of at least 100 spots each of 300 micron diameter or of at least 400 spots each of 150 micron diameter.

14. The array reader of any of the foregoing claims in combination with a carrier (102) for the array comprising a substrate layer (302) carried by a support body (306), said image collection and recording system (140) residing on the same side of the substrate as does the array of features such that the path (P) of said illumination reaches said array (103) before reaching the support body (306), said carrier constructed to absorb excitation radiation penetrating beyond said layer.

15. The array reader of claim 14 in which said support body (306) is transparent, e.g. glass, and between said substrate layer (302) and said transparent body (306) resides a substantially opaque adherent layer (304) capable of substantially blocking excitation radiation tending to enter the transparent body.

16. The array reader of claim 15 in which said substantially opaque layer (304) comprises a layer of metal oxide.

17. The array reader of claim 1 in which said substrate (302) is in the form of a transparent layer carried by a transparent body (306), the image collection and recording system (140) lying beyond the transparent body on the same side of the array as the transparent body.

18. The array reader of any of the foregoing claims in combination with a carrier (102) for said array (103) that comprises an ultra-thin substrate layer (302) on a support body, i.e. the substrate having a thickness less than about 5 micron, preferably less than about 3 micron.

19. The array reader of any of the foregoing claims in which said array (103) is disposed on a substrate (302) comprising a clear layer of nitrocellulose or polystyrene.

20. The array reader of any of the foregoing claims 1-18 in which said substrate (302) is a nitrocellulose membrane that is porous at least in its outer region.

21. The array reader of any of the foregoing claims in combination with a substrate (302) carrying excitation energy reference features (166) distributed across said two-dimensional array of features, said image collection and recording system (140) including a normalizing arrangement (see Fig. 10) for normalizing data detected in the vicinity of respective reference features based on the quantity of detected emission from the respective reference features.

22. The array reader of any of the foregoing claims in which said illumination system (120) comprises at least two different light source sub-systems (402, 412, 406; 404, 414, 408, see Fig. 7A) respectively of substantially different wavelengths, each associated with a respective optical system delivering light along a path, the paths of said sub-systems to said substrate lying along respectively different axes, the axes being spaced apart about said substrate.

23. The array reader of claim 22 having two said different light source subsystems the paths of which are disposed on diametrically opposite positions about said substrate (Fig. 7A.

24. The array reader of any of the foregoing claims in which said illuminating system includes light sources (e.g. diodes 402, 404) selected respectively

to excite Cy3 and Cy5, and said image collection and recording system (140) includes changeable band-pass filters (424, 424', Fig. 7A) suitable to permit passage of emissions respectively from Cy3 and Cy5 or a single band-pass filter (424) is provided suitable to permit multiple band-pass emission such as both band-pass emissions of Cy3 and Cy5.

25. The array reader of any of the foregoing claims in which the image collection and recording system (140) is adjustable between at least two settings, the first and second settings constructed and arranged respectively to form a single image of an array format of dimensions 6.5 mm x 9.0 mm (504, Fig. 8) and of an array format comprising two separated sub-windows, each of dimensions 4.5 mm x 4.5 mm disposed within a 4.5x 13.5 mm rectangle (502, Fig. 8).

26. The array reader of any of the foregoing claims in which said illumination system includes a diode light source (132) and a homogenizer (130, Fig. 2B) effective to reduce variation in flux density across the field of illumination.

27. The array reader of claim 26 in which said homogenizer (130) comprises an elongated transparent, internally reflective rod, which may be straight or curved and may have round, square or rectangular cross section, and be twisted or untwisted.

28. The array reader of any of the foregoing claims in which said image collection and recording system (140) is constructed and arranged to resolve the image on said solid state array (203, Fig. 4) at resolution no finer than about 10 micron.

29. The array reader of claim 28 in which said resolution is between about 12 and 15 micron.

30. The array reader of any of the foregoing claims in which said image collection and recording system (140) includes an interference filter (424, Fig. 7A), collection optics (422) of said system preceding said filter constructed to direct

collected rays in parallel to said filter, and imaging optics (422') constructed to image parallel rays leaving said filter upon said solid state sensor (420).

31. The reader of any of the foregoing claim for use with an array support which holds more than one array, and wherein the reader is constructed and arranged to read and process each array as an independent array.

32. A method of conducting an assay comprising preparing a two-dimensional spotted array of amino or nucleic acid features on a substrate, preferably by spotting liquid samples thereon, in which features throughout the array carry photo-responsive markers and employing the reader of any of the foregoing claims to read the array.

33. The method of claim 32 in which the assay is a diagnostic immuno assay based on protein derived from blood.

34. The method of claim 32 in which the immunoassay is of an antibody capture configuration, employing immobilized antibodies.

35. The method of claim 34 adapted to detect or monitor for malignant cancer.

36. The method of claim 35 adapted to detect ovarian cancer for initial diagnosis or to monitor patients at risk for relapse.

37. The method of any of the claims 32 to 36 in which the substrate is disposed within a sealed disposable bio-cassette (4, Fig. 7B) and imaging is performed through a transparent window visually accessing said substrate, or a transparent body forms a side of said bio-cassette and carries said substrate, said substrate being transparent, such as an ultra-thin nitrocellulose or polystyrene layer, and said array is accessed visually by said array reader through said transparent body and through said substrate.

38. A method employing the array reader of any of the array reader claims, or any of the foregoing method claims, for reading an array on a substrate, in which the array includes intensity calibration features (164) of fluorescing character generally proportional in emission intensity to their illumination over the range of operable illumination intensities, including, forming an image of the array employing said array reader, and normalizing recoded array data (e.g. for spots 166, Fig. 4C) based on quantitative data acquired from nearby intensity calibration features.

39. A fluorescence reader-based diagnostic method for a disease for which there is a set of known protein biomarkers in blood or other body constituent, comprising the steps of (1) providing a two-dimensional array (103) of different reagents on a substrate, the reagents respectively specific to bind members of a set of said biomarkers capable of diagnosing the disease, (2) exposing the array to fluorophore-labeled blood or body-constituent extract of an individual containing the biomarkers if present in the individual's blood or body constituent, (3) while the array is stationary, exciting the array by simultaneously illuminating the entire two-dimensional array by light (e.g. by 120, Fig. 1) at fluorophore-excitation wavelength employing dark field illumination, (4) capturing a fluorescence image of the entire two-dimensional excited array on a single frame of an imager comprising a solid state array, e.g. by 140, Fig. 1) and (5) analyzing the fluorescence image for the presence of the disease (e.g. by computer 104, Fig. 1).

40. The method of claim 39 in which the step of simultaneously illuminating the entire two-dimensional array is carried out by directing excitation radiation from a diode (122, 132, 402, 404) or set of diodes to produce illumination at a wavelength selected to excite said fluorophore, at a power density of at least $30mW/cm^2$.

41. The method of claim 39 or 40 in which fluorescence intensity reference features (164) are distributed through the array and the detected radiation from said biomarkers is normalized by the reader based on the response of said references to said illumination.

42. The method of any of the foregoing method claims 32-41 in which at least 50 pixels of a solid-state sensor represent the image of a feature of the array.

43. The method of any of the preceding diagnostic method claims 39-42 in which the biomarkers attach to antibodies.

44. The method of any of the preceding diagnostic method claims 39-43 in which the array is formed to immobilize protein biomarkers selected to diagnose presence of ovarian cancer.

45. A method of reading an array on a substrate having features that include fluorophores, in which the array includes intensity calibration features of fluorescing character generally proportional in emission intensity to their illumination over the range of operable illumination intensities, including, forming an image of the array employing an array reader, and normalizing recoded array data during the reading of the array from nearby intensity calibration features within the array.

46. The method of claim 45 adapted to perform diagnosis for a disease for which there is a set of known protein biomarkers in blood or other body constituent, comprising the steps of (1) providing a two-dimensional array of different reagents on a substrate, the reagents respectively specific to bind members of a set of said biomarkers capable of diagnosing the disease, and including with the array said intensity calibration features, (2) exposing the array to fluorophore-labeled blood or body-constituent extract of an individual containing the biomarkers if present in the individual's blood or body constituent, (3) while the array is stationary, exciting the array by simultaneously illuminating the entire two-dimensional array by light at fluorophore-excitation wavelength employing dark field illumination, (4) capturing a fluorescence image of the entire two-dimensional excited array on a single frame of an imager comprising a solid state array, (5) normalizing the recorded array data based on said calibration features in the array and (6) analyzing the fluorescence image for the presence of the disease.

47. The method of claim 45 or 46 in which the entire two-dimensional array is illuminated for forming said image by directing excitation radiation from a diode or set of diodes to produce illumination at a wavelength selected to excite said fluorophore, at a power density of at least $30mW/cm^2$.

48. The method of any of the foregoing method claims 45-47 in which at least 50 pixels of a solid-state sensor represent the image of a feature of the array.

49. The method of any of the preceding employed to perform a diagnosis in which features of the array include antibodies.

50. The method of claim 49 in which the features of the array are selected to diagnose presence of ovarian cancer.